ASSESSMENT OF ALKALINE PHOSPHATASE AND BONE SIALOPROTEIN LEVEL IN ADOLESCENTS UNDERGOING BONE BORNE IMPLANT SUPPORTED RPE – A PILOT STUDY

Akshay Mohan¹, S. Harish Babu²

¹Post Graduate Student, Department of Orthodontics and Dentofacial Orthopedics, Saveetha Dental College and Hospitals, Saveetha Institute Of Medical and Technical Sciences, Saveetha University, Chennai, India
²Professor, Department of Orthodontics and Dentofacial Orthopedics, Saveetha Dental College and Hospitals, Saveetha Institute Of Medical and Technical Sciences, Saveetha University, Chennai, India

Corresponding author-
Post Graduate Student, Department of Orthodontics and Dentofacial Orthopedics, Saveetha Dental College and Hospitals, Saveetha Institute Of Medical and Technical Sciences, Saveetha University, Chennai, India
PIN- 600077.
Mobile number- +91 8867596565
E-mail id- akshv94@yahoo.co.in

ABSTRACT
Aim: To evaluate the level of new bone formation using molecular markers along with radiographic evidence of palatal expansion using bone borne implant supported rapid palatal expander.
Objective: To assess the level of alkaline phosphatase, bone sialoproteins from GCF in adolescents undergoing expansion with bone borne implant supported rapid palatal expansion.
Design: This was a prospective clinical trial study
Setting: The study was conducted in Department of Orthodontics and Dentofacial Orthopedics
Methods: This pilot study involved assessment of alkaline phosphatase and bone sialoprotein from GCF in patients undergoing bone borne implant supported rapid palatal expansion at the Department of Orthodontics and Dentofacial Orthopedics. Inclusion criteria involves patients in the late adolescents group with unilateral or bilateral constricted upper arch, and having midpalate suture in Stage C or D or E.
Results: The net difference between baseline T0 and T3 (28 days) values of concentration of alkaline phosphatase, bone sialoprotein were 197.87 ng/dL and 112.22 ng/dL respectively. With radiographic evidence, evidence of expansion is approximately 3.772 ± 3.16 mm. There was no statistical significance difference between baseline and post values however clinical correlation suggests a possible significant association.
Conclusion: Correlating the clinical measurements, radiographic measurements and concentration of molecular markers it can be concluded that bone borne implant supported rapid palatal expansion causes new bone formation in the expanded maxillary palatal segment which is clinically significant but statistically insignificant.
Keywords: Cone-Beam Computerized Tomography, Maxillary Expansion, Orthodontics, Palate.

INTRODUCTION
Rapid maxillary expansion has been a treatment option in patients with transverse discrepancies and a method of achieving skeletal expansion in prepubertal patients up to early adolescence[1]. With the modus operandi of disrupting mid palatine and circum maxillary sutures, many RME appliances have managed to achieve transverse maxillary expansion along with an increase in nasal airway. Achieving these changes is not without concomitant changes such as alveolar bone bending, buccal inclination of posterior teeth and unfavorable periodontal changes in some cases. Mini implants have proven to be a versatile anchorage device to provide bone borne anchorage in treatment of transverse discrepancies [2]. Miniscrew assisted rapid palatal expansion has now made it possible to treat transverse discrepancies despite age and with minimal detrimental changes to posterior teeth [3]. The challenge faced in miniscrew assisted rapid palatal expansion is to reduce the resistance offered by the sutures without causing failure of mini-implants. Resistance offered by sutures will lead to increased loading on the mini-implants during activation leading to pain and failure of the mini-implant. Major areas of resistance to MARPE are mid palatine suture and the pterygomaxillary articulation[4][5]. With increasing age, the interdigitation at sutures increases making the site more rigid[6][7].
To monitor orthodontic tooth movement noninvasively in human beings, changes have been examined in the patient profile, the levels of various enzymes, cytokines, growth factors, biomarkers and proteoglycans in gingival...
crevicular fluid and saliva. Among those components that change and respond to orthodontic force, ALP, TRAP, LDH, and aspartate aminotransferase (AST)[8,9]. Although the clinical and radiographic follow-up examination remains the basis for patient’s evaluation, an investigation of saliva, that is a fluid that contains local and systemically derived markers, may provide the basis for a phase-specific screening of orthodontic tooth movement[10]. The increase in osteoblastic activity during bone formation will be accompanied by an increased expression of an enzyme called alkaline phosphatase[11]. Gingival crevicular fluid (GCF) is a transudate with constituents from a variety of sources, including microbial dental plaque, host tissues, and serum, with a high site specificity[12]. A number of GCF constituents including host enzymes have been proposed as diagnostic indicators of periodontal status[13]. Among these enzymes, one of the first to be identified was alkaline phosphatase (ALP). GCF ALP has a primary role in bone mineralization, and it has been shown to be sensitive[14] to alveolar bone formation during orthodontic tooth movement. In this study, bone remodeling pattern of mid palatal suture and palate was assessed by ALP estimation from palatal tissue and GCF. To date, only a few studies have investigated the levels of GCF constituents during maxillary expansion[14][15][16].

The Human Alkaline Phosphatase, ALP GENLISA™ ELISA kit was used as an analytical tool for quantitative determination of Human Alkaline Phosphatase, ALP in GCF from the palatal. The Bone Sialoprotein (BSP) ELISA Assay Kit was used for quantitative determination of Bone sialoprotein from GCF. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies were used in this kit. Because of the small amount of data available on the metabolic changes at the palatal (tension) alveolar sites during the retention phase after bone borne implant supported rapid palatal expansion treatment, the aim of this prospective clinical study in adolescent subjects was to monitor cancellous bone formation at the tension sites of the mid palatal suture region.

Many studies have used rapid palatal expanders for maxillary expansion, but no study till date has assessed and correlated molecular biology of new bone formation with radiographic and clinical evidence of expansion. The aim of the present study was to prove new bone formation before and after bone borne implant supported rapid palatal expansion quantified using alkaline phosphatase and bone sialoprotein from GCF and relating the same with radiographic and clinical measurements suggesting palatal expansion.

**MATERIALS AND METHODS**

This pilot study involved 12 subjects (mean age: 21.4 ± 5.5 years; range: 14-28 years; 7 females, 5 males) treated at the Department of Orthodontics and Dentofacial Orthopedics for bone borne rapid palatal expansion. This study design was approved by the institutional ethical committee (SDC/SIHEC/2020/DIASDATA/0619-0220). Inclusion criteria involves patients in the late adolescents group with constricted upper arch with unilateral or bilateral crossbite, and having midpalatal suture in Stage C or D or E. Exclusion criteria involved individuals who had developmental and craniofacial anomalies, compliance problems, temporomandibular joint disorder, active periodontal diseases and caries, systemic diseases and history of previous orthodontic treatment. Consent was obtained from the patients after they received detailed information about the clinical trial. Pre-operative models were obtained and intermolar width and palatal index were calculated to obtain Korkhaus index measurements. Samples whose values were less than 42% were included in the study. Ashley Howe's index and Ponts index were also carried out for further identification of the need of transverse expansion.

*Sample collection (baseline) T0*

Sample collection of GCF using Eppendorf tubes was performed immediately before the beginning of the treatment by bone borne implant supported rapid palatal expander.

*Sample collection (subsequent) T1-T3*

Sample collection for subsequent appointments was performed using gingival crevicular fluid collection from the crevicular sulcus of the left and right first molar tooth of maxillary arch on the 14 th, 21st and 28 th day.

*Sample processing*

All collected samples were stored in 98ul of 10% Phosphate Buffer saline (pH=7.4) and 2ul of protease inhibitor enzyme and transferred to the cell Lab of the University within 10 minutes for immediate processing.

*Orthodontic Protocol*

The bone borne implant supported rapid palatal expander was used for maxillary expansion which was activated 1mm a day for 10 days. Post expansion the expander was then locked and retained for 6 months. Treatment plan
and patient selection was provided by four providers (HB, and AM). The protocol formation for evaluation of molecular markers was done by AM. Patients did not receive any orthodontic treatment during the study period; however, all problems apart from the constricted maxilla problems were addressed after the completion of the present study.

*Elisa Methodology*

Manufacturer's instructions were followed for GCF protocol to assess human Alkaline phosphatase and bone sialoprotein at baseline while subsequent values were analyzed and preserved with protease inhibitor. The analysis was done digitally to avoid manual error and the values were in terms of ng/dl. The two examiners (A.M) and (H.B) analyzed the results of pre and post expansion GCF samples.

**STATISTICAL ANALYSIS**

All data collected during the present study was interpreted as mean and standard deviation which was assessed using Statistical Package for Social Sciences version 20.0 (SPSS Inc., Chicago, IL, USA). There is no statistical significance between age, gender and change in clinical, radiographic measurements. The mean concentration of activity of enzyme alkaline phosphatase, bone sialoprotein was analyzed using a non-parametric test namely Kruskal Wallis to assess statistical significance from T0 and T3.

**RESULTS**

The mean, standard deviation and significance of the ALP and bone sialoproteins levels observed in pre and post expansion samples were assessed. No statistical significance or association was found between values of molecular markers, clinical measurements, radiographic measurements (where p-value<0.05 was considered statistically significant). 21.4 ± 5.5 years; range: 14-28 years; 7 females, 5 males.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value (Mean ± SD)</th>
<th>Significance (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age(years)</td>
<td>21.4 ± 5.5</td>
<td>p=0.97</td>
</tr>
<tr>
<td><strong>Molecular markers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase(ng/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>68.84</td>
<td>p = 0.213</td>
</tr>
<tr>
<td>T1</td>
<td>104.61</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>171.82</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>266.71</td>
<td></td>
</tr>
<tr>
<td>Bone sialoprotein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>16.66</td>
<td>p=0.270</td>
</tr>
<tr>
<td>T1</td>
<td>44.78</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>77.44</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>128.88</td>
<td></td>
</tr>
<tr>
<td>Clinical measurements(mm)</td>
<td>4.20 ± 3.47</td>
<td>p=0.266</td>
</tr>
<tr>
<td>Radiographic measurements(mm)</td>
<td>3.772 ± 3.16</td>
<td>p=0.213</td>
</tr>
</tbody>
</table>

Table 1 illustrates tabular description of change in molecular marker(ng/dl), difference in clinical measurements(mm) and difference in radiographic measurements taken at different time intervals (T0-baseline to T3-28 days) with orthodontic expansion at the rate of 1 mm/ day for 10 days. No statistical significance or association was found between values of molecular markers, clinical measurements, radiographic measurements (where p-value<0.05 was considered statistically significant).
Graphical illustration depicts change in concentration of alkaline phosphatase and bone sialoproteins (ng/ml) over time (baseline T0, T1, T2, T3) after activation of bone borne implant supported rapid palatal expander for maxillary expansion. X axis depicts time in days; Y axis depicts concentration of molecular markers in ng/ml. No statistical significance or association was found while using Kruskal Wallis test (p>0.05).

**DISCUSSION**

In this study, we assessed alkaline phosphatase and bone sialoprotein activity up to 28 days after bone borne implant RPE to evaluate alveolar bone formation at the tension sites of mid palate. The results show significant increases of alkaline phosphatase and bone sialoprotein activity at T3 (28th day) without clinically relevant tissue inflammation. To the author’s knowledge, no clinical trial using GCF samples from pre operative and post expansion has been conducted to assess the ALP and bone sialoproteins levels in the mid palatal suture region after bone borne implant supported RPE expansion. Therefore, the purpose of this study was to investigate and compare the ALP and bone sialoproteins levels of mid palatal suture after expansion with bone borne implant supported RPE.

Generally, many researchers have described the role of biomarkers during orthodontic force application such as ALP and LDH salivary enzymes activities, which have been associated with the bone remodeling process, but this is the first ever research done to describe the trend of activity of ALP expression in the palatal tissue in patients undergoing bone borne implant supported RPE. The ALP is a host enzyme that allows bone mineralization by hydrolyzing inorganic pyrophosphate, and it has been extensively correlated with the rate of bone formation[17]. Alkaline phosphatase and bone sialoprotein are very important enzymes, and it is considered as a part of normal turnover of periodontal membrane, cementum, and bone, because it is produced by many cells. The trend of change in alkaline phosphatase and bone sialoprotein depicts active bone growth during appliance activation. On comparing baseline to T3 values the difference in concentration was almost 4 times higher suggesting bone deposition with time.
While the level of alkaline phosphatase and bone sialoprotein enzymes activities showed highly significant differences from T0 to T3, these findings demonstrated that there were overlaps between bone destruction and bone formation processes during expansion and our result is in accordance with the basic principle of bone remodeling process that stated a bone remodeling is carried out by a functional and anatomic structure known as the basic multicellular bone unit that works in a coordinated overlapping manner. It can be concluded from the results of the current study that ALP and bone sialoprotein activity when taken with radiographic changes in bone morphology appears to be affected by mechanical forces generated by the bone borne implant supported RPE that can cause a bone remodeling process around the mid palatal suture and at the growth center in the temporomandibular joint. Although these enzymes might be fluctuated by factors other than orthodontic and orthopedic forces such as gingival and periodontal inflammation, and oral hygiene of the subjects involved, these other factors were kept under control during the course of the study. Furthermore, the ALP and bone sialoprotein activity can also be increased during rapid growth phases of childhood such as late infancy and early puberty. Hence the subjects involved in the current study were in the mid palatal suture staging of C, D and E. ALP and bone sialoprotein can be promising bone remodeling biomarkers to assess the biological alterations and improvement in the bone remodeling process that occurs during treatment with bone borne implant supported RPE. Animal studies have shown that the bone remodeling cycle begins with an early wave of resorption, which requires 3 to 5 days, followed by its reversal (5-7 days) and a late wave of bone formation that continues for 7 to 14 days. This process would occur at both the tension and compression stress sites. Moreover, a similar process has been described for human bone, where bone formation appears to begin 10 days or 3 weeks after the initial resorption.

CONCLUSION
Correlating the clinical measurements, radiographic measurements and concentration of molecular markers it can be concluded that bone borne implant supported rapid palatal expansion causes new bone formation in the expanded maxillary palatal segment which is clinically significant but statistically insignificant.

LIMITATIONS
All obtained values and interpretation must be taken with some amount of caution as there was heterogeneity in the method of collection of baseline and subsequent samples. The limitation of the present study is its small sample size. A study with a larger sample size will be required to confirm these findings.

REFERENCES


